Title of the article: Investigation of the relationship between salivary cortisol, training load and subjective markers of recovery in elite Rugby Union players

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Abstract

Purpose: Insufficient recovery can lead to a decrease in performance and increase the risk of injury and illness. The aim of this study was to evaluate salivary cortisol as a marker of recovery in elite Rugby Union players. **Method:** Over a 10-week pre-season training period, 19 male elite Rugby Union players provided saliva swabs bi-weekly (Monday and Friday morning). Subjective markers of recovery were collected every morning of each training day. Session Rating of Perceived Exertion (sRPE) was taken after every training session and training load was calculated (sRPE x session duration). **Results:** Multi-level analysis found no significant association between salivary cortisol and training load or subjective markers of recovery (all, $p>0.05$), over the training period. Compared to baseline (week 1), Monday salivary cortisol significantly increased in weeks 4 (14.94 ± 7.73 ng.ml; $p=0.04$), 8 (16.39 ± 9.53 ng.ml; $p=0.01$) and 9 (15.41 ± 9.82 ng.ml; $p=0.02$) and Friday salivary cortisol significantly increased in weeks 5 (14.81 ± 8.74 ng.ml; $p=0.04$) and 10 (15.36 ± 11. 30 ng.ml; $p=0.03$). **Conclusions:** The significant increase in salivary cortisol on certain Mondays may indicate players did not physically recover from the previous week of training or match at the weekend. The increased Friday cortisol levels and subjective marker of perceived fatigue indicated increased physiological stress from the weeks training. Regular monitoring of salivary cortisol combined with appropriate planning of training load, may allow sufficient recovery, to optimise training performance.

Key words:
Readiness to Train, Physiological Stress, Internal Load, Monitoring Markers, Salivary Hormones

Introduction

Elite athletes are under considerable physiological stress due to high levels of training and performance requirements. [1] Increased stress can have negative effects on performance, particularly if there is an imbalance between training load and recovery. [2] Insufficient recovery can lead to a decrease in performance and may lead to non-functional overreaching or overtraining, while also increasing the risk of injury and illness. [2] Training load has been widely used as a monitoring marker to optimise training in many teams' sports such as Rugby, [3] and Australian football. [4] Evidence suggests that with just a 1-week increase or "spike" in training load, players are more susceptible to injury. [5]

Monitoring markers are imperative to ensure sufficient recovery, manage stress (both physiological and psychological), and optimise training for peak performance. [5, 6] Cortisol is a stress hormone found in saliva, serum (blood) and urine. Salivary cortisol has been found to be a marker of physiological stress and may provide an understanding of physiological response from training and matches in team sports. [7, 8, 9, 10] Saliva collection is non-invasive, time efficient and easy to collect, meaning it can be used in an applied setting. [11] Despite this, limited research has previously evaluated the effectiveness of measuring weekly salivary cortisol as a monitoring marker in terms of identifying recovery state in Rugby Union players. [12] To gain a better understanding of the players' recovery, both objective (internal and external) and subjective markers should be used. [13, 14] There is currently a dearth of scientific research investigating the relationship between salivary cortisol, training load and subjective markers of recovery in Rugby Union.

Stress can be both psychological and physiological, however for the purpose of the current study physiological stress has been defined as internal or external forces or stressors, which alters the dynamic equilibrium or homeostasis of the body. [15] Recovery has been defined as the ability to meet or exceed performance for a particular activity. [16] However, for specificity to the current study recovery is the return of salivary cortisol to baseline levels or above.

The frequency and investigation of the effects of training compared to weekly salivary cortisol, in elite Rugby Union players for saliva collection are limiting factors in previous research. [11, 17] Agostinho et al. [18] study with judo athletes found that training load did not influence a change in salivary cortisol levels, even with significant increases in training load. However, the study was conducted over 19-weeks with only six testing time points of saliva. Similarly, Nunes et al. [19] found that salivary cortisol did not change even with fluctuations in internal training load in elite female basketball players. Again, salivary cortisol testing was infrequent, with saliva collection only pre and post the 12-weeks of the study. Cormack et al. [9] conducted a study in Australian Rules Football (AFL) over a 22-week period with 20 testing time points. The results found that an increase in salivary cortisol and decrease in countermovement jump (CMJ) height indicated players had incomplete recovery of neuromuscular (decreased force production) and hormonal status, which may lead to a catabolic state. However, the study did not conduct statistical analysis comparing weekly training volume and salivary cortisol. Additionally, training was calculated as training volume and not training load (sRPE x session duration). Training load has been found to be more valid and reliable measure of training response, than training volume as it takes into account players' internal load. [3] Cunniffe et al. [8] conducted an 11-month longitudinal saliva study with Rugby Union players, however there were only seven testing time points over this period. Other studies only examined the acute effect of a Rugby match on salivary cortisol as a marker of recovery, with post-match salivary collection ranging from hours to 6-days. [7, 20, 21] More frequent testing of salivary cortisol can provide more accurate results [11] and the use of a standardised testing day facilitates the evaluation of weekly variations over a period of time.

Another limiting factor in previous research is the method standardisation for saliva collection. [11, 17] Moreira et al. [22] study with futsal players took saliva swabs weekly over a 4-week period of intensified training. The results found no changes in salivary cortisol even with significant changes in training load, however this did not account for a normal training schedule. Rowell et al. [23] found as internal load increased salivary cortisol levels also significantly increased, in professional soccer players, over an entire soccer season. However, no baseline measures were taken, and sleep or stressful situations were not recorded. Additionally, no statement of pre-saliva sample collection (e.g. no brushing of teeth, no caffeinated drinks consumed), or any indication of consideration for diurnal variations, [17] as player may have woken 30-mintues or 2-hours prior to the collection. To reduce measurement error and ensure a stringent method for salivary cortisol, the players' diet before the swab, sleep the night before, physical activity, any stressful situations and diurnal variation must be taken into account and recorded. [8, 17, 24] However, most studies [8, 7, 20, 21, 18, 19, 22, 23] do not account for all the factors, increasing the risk of variability. [17] Research is therefore needed to investigate the association between weekly salivary cortisol and training load,

[18] using a stringent method for saliva collection, [14] in Rugby Union players.

To the authors' knowledge, no study has been previously published examining weekly salivary cortisol responses in Rugby Union players. The main aim of this study was to explore the association between weekly training load, resting salivary cortisol (objective marker of recovery) and subjective markers of recovery. This will bridge the gap in research and provide practically applied research.

Method

Subjects

Nineteen male elite Rugby players volunteered to take part in the study (age 19.7 ±1.1 years, height 184.5-±7.7 cm, body mass 96.2 ± 12.5 kg). All players were contracted with the Academy of a professional Rugby team and trained full-time with the Academy or Senior team. All training was planned and scheduled by the coaches and adjusted where they saw fit. This included the download week (week 3), which was a known as a 'recovery week'. Players were away from the training facilities but were prescribed sessions by coaches to complete.

Each week typically consisted of 4-5 days a week, approximately 10 sessions a week (Table 2), with multiple sessions a day. Sessions included gym/resistance, Rugby sessions; skills based sessions (e.g. passing, tackling, lineout's) and conditioning pitch sessions. All gym sessions were completed with the Academy team in the morning, approximately 3-4 sessions a week.

All players were informed of the study requirements and provided written informed consent. The study was approved by the University Research Ethics Committee and all procedures were in accordance with the Declaration of Helsinki.

Design

The study was completed over 10-weeks (pre-season) with 19 players, to investigate the physiological stress response to training. Salivary cortisol was compared to training load and subjective monitoring markers of recovery.

Together with saliva collection, the monitoring included a number of subjective markers of recovery (Table 1) and training load variables. Swabs were collected on a Monday, which coincided with the start of the training week, to provide an indication of the player's recovery from the previous week of training or match at the weekend (Table 2). Friday saliva collection was the last day of the training week, investigating the effect of the week's training and/or recovery state for a match at the weekend.

Methodology

All testing took place in the Rugby team's training facilities, located on the University campus, to ensure minimal disruption to training and continuity with the players' normal training schedule. Data collection for both saliva and subjective markers of recovery took 20-minutes to complete each morning. The players prepared their own snacks and pre-gym breakfast with advice from the qualified team nutritionist.

Baseline measures

On the first week of players returning to training, which was a medical screening week with low training load scheduled, saliva samples were collected each morning for 4-days; Monday, Tuesday, Thursday and Friday (in keeping with the player's normal training schedule). The average of the 4-days was calculated for baseline data to account for the individual variations and effects of sleep and stressful situations. [24] All saliva samples (Monday and Friday) were compared to the average of week 1 baseline data.

Pre-season testing

Players' saliva samples and a self-reported sleep diary were collected twice a week, on a Monday and Friday morning prior to training. Prior to the Monday swab testing, players had a least one full day of recovery from training or playing a match. Subjective markers of recovery (Table 1) were collected each morning of a training day. Session rate of perceived exertion (sRPE) was taken after every training session.

The sleep diary recorded the players sleep quantity (time in hours/minutes), how long it took them to fall asleep, if they woke during the night and how long for and sleep quality on a 0-4 Likert scale, 0=very good (very sound) and 4=very bad (restless). The sleep diary is similar to the sleep diary and sleep questionnaire in previous research. [25, 26] Sleep data were documented as it has been found that sleep can affect salivary cortisol levels. [24]

Saliva collection protocol

Players' saliva samples were collected within 1-hour of the players waking up, between 7-8am; this was to account for diurnal variation. [17] Cortisol levels increase upon wakening and start to decrease an hour after wakening. [17] This method was used instead of a set time, as players wakening times differed.

To ensure more stringent testing and reduce salivary cortisol measurement error; [17] players were required to have eaten breakfast, refrain from brushing their teeth and eating chewing gum. They were also told to avoid drinking any caffeinated drinks (tea, coffee or sports drinks) or consuming alcohol 24-hours prior to testing. [8] Research has found that sleep and stressful situations can affect salivary cortisol results. [24] Each player recorded, what they had eaten for breakfast, how they slept the previous night and any stressful situations the night before or that morning.

Players placed the oral fluid collector (OFC) swab (Soma Bioscience, Wallingford, UK) on their tongue and closed their mouth. They did not suck or move the swab around their mouth to ensure the test was consistent and reduced variability. [27] The indicator on the stem turned blue when the sample was complete (swab collected 0.5ml oral fluid). The swab was then placed in the OFC buffer bottle of assays (sodium phosphate, salts, detergents and preservatives).

The researcher gently mixed the samples in the OFC buffer bottle for 2-minutes. Two drops of the sample were added to the sample window of the lateral flow device (LFD) and left for exactly 15-minutes ('incubation' phase). The strip was placed in the LFD real-time reader with results ready within 22-seconds. Cortisol units were recorded as ng.ml. Soma Bioscience OFC collectors have been validated against ELISA and have been proven a reliable method to collect and analyse salivary cortisol. [28]

Training load

To subjectively measure the player's exercise intensity from the session, sRPE was recorded after every training session, [3] using the modified Borg's 0-10 scale. The players were asked after each training session 'how intense do you felt the session was?' [29] RPE has been found to be a valid and reliable monitoring marker for internal load and exercise intensity, compared to heart rate and blood concentrations. [3]

Training load for each session was calculated by sRPE x duration of session (minutes). [29] Session training load was added together to provide weekly training load data. The weekly training load included all training sessions and matches (academy or senior) played during the week.

Statistical analysis

Descriptive statistics were calculated, using MLwin software (version 2.36), for all variables. Non-parametric analysis was used, as data were not normally distributed. Natural log transformation was used to calculate salivary cortisol means, due to the variability in salivary cortisol. Significance was set at $p<0.05$.

Multi-level analysis was conducted using MLwin. Multi-level modelling was used as there were multiple testing time points, and the study sought to investigate both between and within subject variability. A two-level model was conducted, training weeks (level 1) and players (level 2), to investigate the variance between weeks and players and the variance within players across those training weeks.

Results

Figure 1 shows the weekly mean ± standard error (SE) of training load and salivary cortisol on a Monday and Friday morning across the 10-week training period.

The multi-level analysis found no significant association ($p>0.05$) between Monday cortisol and the previous weeks training load (0.00028 (0.00082 ng.ml) (beta (SE)) or between Friday cortisol and the same weeks training load (0.00108 (0.00072) ng.ml) (Table 3). No significant association ($p>0.05$) was found between salivary cortisol and the subjective markers of recovery (perceived fatigue, muscle soreness, stress level, energy and physical recovery) (Table 4).

Compared to baseline (week 1), Monday cortisol significantly increased in week 4 ((4.54842 (2.19724) ng.ml $p=0.04$)), week 8 ((5.97474 (2.19724) ng.ml, $p=0.01$)), and week 9 ((4.99684 (2.19724) ng.ml, $p=0.02$)) (Figure 1). Friday cortisol significantly increased in Week 5 ((4.39789 (2.17926) ng.ml, $p=0.04$)) and Week 10 ((4.91486 (2.28392) ng.ml, $p=0.03$)) compared to baseline (Figure 1). Friday cortisol levels in week 6 (11.27 ng.ml), week 7 (9.86 ng.ml), week 8 (10.67 ng.ml), and week 9 (7.10 ng.ml) were all close or below baseline levels (10.49 ng.ml). It must be noted Monday cortisol week 8 was collected after a friendly match (Week 7) with 2-days of recovery and week 9 was collected after a competitive match (Week 8) with 1-day recovery. However, no significant difference was found between Monday cortisol results in Week 8 and 9.

Discussion

The aim of this study was to investigate the use of resting salivary cortisol as a marker of recovery in elite Rugby Union players and if there was an association between salivary cortisol, training load and subjective monitoring markers of recovery.

The results from the current study found no significant association between training load and salivary cortisol, when comparing Friday cortisol to the same weeks training load and

Monday cortisol to the previous weeks of training load data. Previous research in futsal, [22] elite female basketball players [19] and judo athletes, [18] also found no significant association between training load and salivary cortisol, even with fluctuations in training load. Guilhem et al. [30] found no correlation between weekly training load and salivary cortisol but reported that salivary cortisol is sensitive to the training season changes (preparation phase, pre-competition and competition) in elite track and field athletes. However, saliva samples were only collected at 8 time points over 4.5-months. Contradictory to these, Rowell et al. [23] study found when internal training load increased salivary cortisol levels also significantly increased, in soccer players. However, no baseline measures were collected and a stringent method was not used when collecting saliva in this study.

No association was found when comparing salivary cortisol to subjective markers of recovery (perceived fatigue, muscle soreness, stress level, energy and physical recovery). Similar to our findings, Guilhem et al. [30] found no significant correlation between the psychological component of fatigue and salivary cortisol. Interestingly, when the subjective marker of perceived fatigue in the current study was analysed weekly, a similar trend to Friday cortisol was observed. Friday cortisol significantly increased in week 5 and 10 (Figure 1), similarly, perceived fatigue levels significantly increased on a Friday in week 5 and 10, compared to baseline (Figure 2). These results suggest that in these weeks, the players' physiological stress increased. However, with the knowledge and expertise of the coaches on training load, they appropriately planned and adjusted training, which may have ensured cortisol levels did not stay elevated and so returned close to baseline. This may have allowed sufficient recovery for optimised training performance. [31]

A potential reason for a lack of association between training load and salivary cortisol and subjective markers of recovery, in the current study, could be large individual variability in salivary cortisol. Previous research has also found large individual variability and unique response for players' cortisol levels, [2, 10, 30] meaning results must be individually assessed. Additionally, sleep and stressful situations were recorded, however, due to the practical nature of the current study ensuring minimal disruption to the players' normal training schedule, sleep and stressful situations could not be controlled.

Monday cortisol levels compared to baseline (10.41 ± 5.09 ng.ml) significantly increased in week 4 (14.94 ± 7.73 ng.ml), which was after the players download week. The increase in salivary cortisol may indicate the players' natural response to the previous training phase, as previous research has found that elevated cortisol levels indicate physiological stress. [7, 21] It would be expected for players to have returned recovered in week 4 after the download week due to prescribed lower training load. However, during the download week, the players were away for the club facilities and non-Rugby related activities may have been engaged with, however these were not recorded. Similarly, in week 8 (16.39 ± 9.53 ng.ml) and 9 (15.41 ± 9.82 ng.ml) salivary cortisol on Monday was significantly higher than baseline. A reason for the elevated salivary cortisol may due to the match played the weekend before (Table 2). These results may indicate that players did not sufficiently recover from the previous week of training or match, as it has been found players' physiological stress can take up to 48-hours to reduce to baseline levels post-match. [7, 21] Interestingly, after the competitive match on Saturday of week 9, the following Monday salivary cortisol levels were not significantly higher than baseline, possibly indicating sufficient recovery. [7, 9] However, only 10 of the 19 players played in the match, which may be the reason for no significant increase in Monday cortisol.

Friday cortisol levels in week 7 (9.86 ± 5.06 ng.ml), week 8 (10.67 ± 9.65 ng.ml), and week 9 (7.10 ± 3.89 ng.ml) were all close or below baseline levels (10.41 ± 5.09 ng.ml) (Figure 1). The reason for the decrease of Friday cortisol (week 7-9) may be due to the coaches having planned and adjusted the players' training programmes to ensure correct preparation for the matches, as the players had both friendly (week 7) and competitive (weeks 8 and 9) matches (Table 2). It must be noted that all 19 players were being prepared for the competitive match in week 9. This adjusted training load is evident in the reduction in Friday cortisol levels. These findings may suggest that appropriately planned training load prior to a match may help ensure reduced physiological stress, to help optimise performance. However, this study did not collect external load, which may add further insight into the Friday cortisol results. Further research needs to explore external load and association with salivary cortisol.

A limitation to the study was baseline measures may represent elevated salivary cortisol levels (heighten stress response) due to collection in week 1 of training instead of the week before, where no training had taken place. This was due to access to the elite players prior to pre-season. Future research should collect baseline measures the week prior to pre-season, to decrease the chance of any physiological stress from training.

Practical application

Salivary cortisol was found to have no association with training load, however it may be a useful internal objective marker to suggest if players have recovered from the previous week of training or a match at the weekend. As fatigue is multi-factorial [13,] which means recovery will have multiple components, combining appropriately planned training load, regular monitoring of salivary cortisol and subjective markers of recovery, would help ensure adequate recovery to optimise performance for training.

This research was conducted over a pre-season period, which may represent a different type of training compared to in-season. Furthermore, matches in the current study were not played weekly which would be the case during the in-season. Future research therefore, is needed to investigate seasonal variations in weekly salivary cortisol over a whole Rugby season, with a larger sample size (entire squad). This would allow further exploration of acute and chronic changes in physiological stress and association with training load. In addition, as salivary cortisol is an expensive marker, further investigation is needed examining the association between training load and subjective markers of recovery to identify if these markers instead could be used.

Conclusion

In conclusion, salivary cortisol may be used as an objective marker of recovery at the beginning of the week, to identify recovery from the previous week of training or match at the weekend. In addition, salivary cortisol may be used as a marker of preparation for a match, by highlighting decreased levels of physiological stress indicating sufficient recovery, which may help to optimise performance. A combination of subjective and objective markers of recovery, including training load, should be used to ensure all aspects of recovery, both physiological and psychological, are accounted for. The combination of markers will provide coaches with sufficient evidence to appropriately tailor training and recovery for the individual player, to optimise performance.

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Figure Captions

Table 1: Morning monitoring markers, collected daily by the team. Likert scale for muscle soreness, stress level, fatigue and stiffness (1= very sore/stress/fatigue, 10= not sore/stress/fatigue). Physical recovery and energy, 1= no energy/not recovery, 10= full of energy/recovered.

Table 2: Training schedule over 10-weeks pre-season period for baseline, download week and matches.

Table 3: Multi-level Regression comparing salivary cortisol on Monday to the previous week training load and Friday salivary cortisol, to the same weeks training load. Cortisol β- beta, SE- standard error, p-value- significance *= $p<0.05$

Table 4: Multi-level Regression comparing cortisol with subjective monitoring markers of recovery. Cortisol β- beta, SE- standard error, p-value- significance *= $p<0.05$

Figure 1: A- Monday Salivary cortisol B-Friday Salivary cortisol, C- training load. Data mean ± SE salivary cortisol (ng.ml) Week 1=baseline, Week 3= download week. * indicates statistical significance ($p<0.05$), ** indicates high statistical significance ($p<0.001$) compared to baseline (Week 1), determined via multi-level analysis.

Figure 2: Weekly variations of Friday subjective fatigue marker, over 10-week training period. Week 5 and 10, significantly increase, week 2, 6 and 8 significantly decreases compared to baseline (week 1). Data mean ± SE salivary cortisol (ng.ml) * indicates statistical significance ($p<0.05$), determined via multi-level analysis.

Table 1. Monitoring markers, collected daily

	Monitoring marker	How it was collected
Subjective (internal)	Muscle soreness	Likert Scale 1-10
	Stress level	Likert Scale 1-10
	Fatigue	Likert Scale 1-10
	Energy	Likert Scale 1-10
	Physical recovery	Likert Scale 1-10 36
	Non-sports stress	Yes/No

Table 2. Training and match schedule

Training Weeks	Training	Matches	Number of players that played (/19)	Overall number of training sessions
1	Baseline			8
2				11
3	Download			7
4				14
5				11
6				10
7		Friendly (Friday evening)	19	8
8		Competitive (Saturday afternoon)	19	7
9		Competitive (Saturday afternoon)	10	10
10				10

Table 3. Multi-level analysis comparing cortisol (Monday and Friday) to training load

	Monday Cortisol to Previous week TL			Friday Cortisol to same week TL		
	Fixed explanatory variables					
Parameter	Estimate	S. Error	P-value	Estimate	S. Error	P-value
Constant	12.85123	0.89644	-	11.81569	0.69525	-
Training Load	0.00028	0.00082	0.73	0.00108	0.00072	0.13
Level 2 (between Players) Variance	9.58	5.00	-	3.81	3.04	-
Level 1 (within players) Variance	50.20	5.82	-	50.19	5.63	-

Table 4. Salivary cortisol multi-level analysis, to subjective monitoring markers of recovery

	Salivary Cortisol		
	Fixed explanatory variables		
Parameter	Estimate	S. Error	P-value
Constant	11.66416	0.73796	-
Fatigue	0.08295	0.49141	0.87
Muscle soreness	-0.02745	0.44126	0.95
Stress level	0.21972	0.39418	0.58
Energy	0.07421	0.40575	0.85
Physical recovery	-0.0559	0.15656	0.72
Level 2 (between Players) Variance	7.14	3.37	-
Level 1 (within players) Variance	32.05	3.47	-

Figure 1. Weekly variations in salivary cortisol and training load, over 10-week period

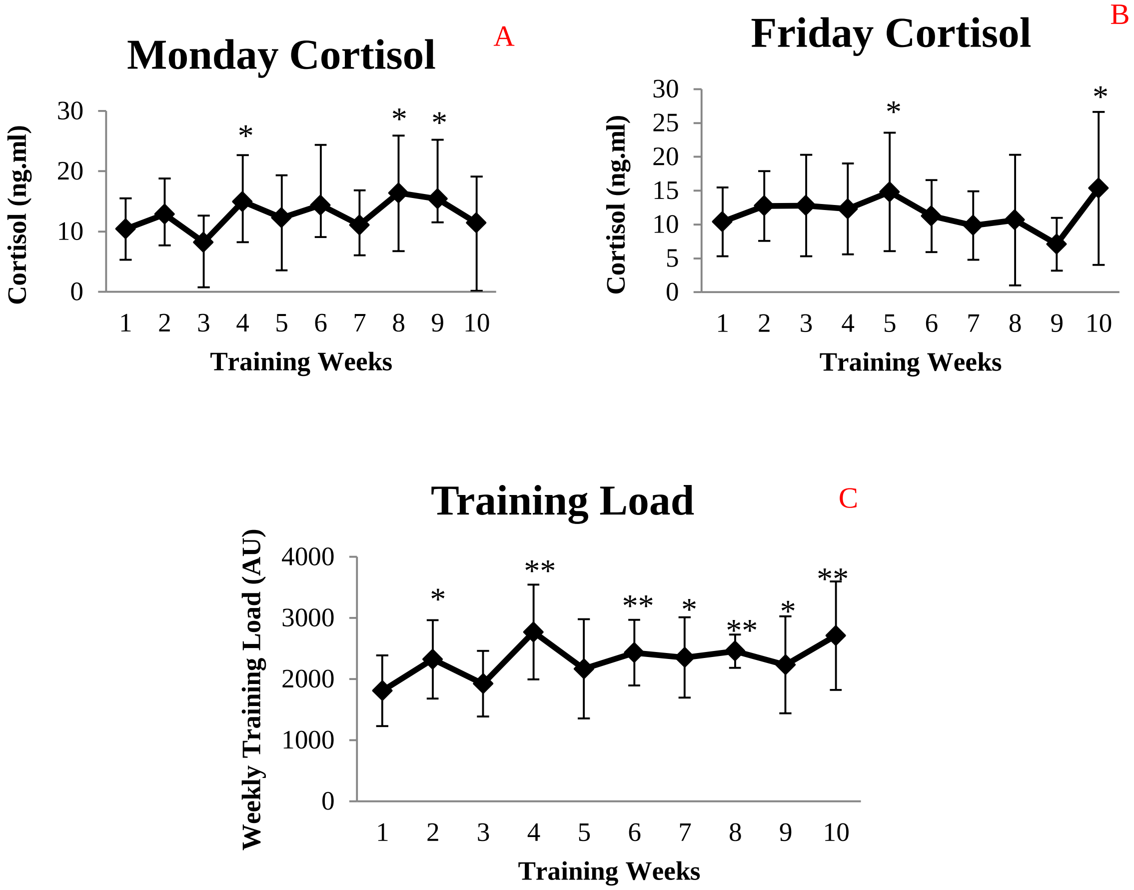

Figure 2. Weekly variations in subjective fatigue (Friday) across the 10-week training period